PATENT COOPERATION TREATY

TRANSLATION

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	See form PCT/ISA/210
Applicant's or agent's file reference: CP61158PCT	FOR FURTHER ACTION: See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/FR2004/002955	19.11.2004	19.11.2003

International Patent Classification (IPC) or both national classification and IPC
C12N15/30, C07K14/44, C12N1/11

Applicant
INSTITUT DE RECHERCHE POUR LE DEVELOPPEMENT (IRD)

1. This opinion contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/EP	Authorized officer
Facsimile No.	Telephone No.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/FR2004/002955

Box No. I Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This opinion has been established on the basis of a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of international search (under Rule 12.3 and 23.1(b)).

2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

☒ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☒ in written format

☒ in computer readable form

c. time of filing/furnishing

☐ contained in the international application as filed.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/FR2004/002955

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-9	YES
	Claims		NO
Inventive step (IS)	Claims		YES
	Claims	1-9	NO
Industrial applicability (IA)	Claims	1-9	YES
	Claims		NO

2. Citations and explanations:

Reference is made to the following documents:

D1: LOHMAN KL ET AL: "Molecular cloning and characterization of the immunologically protective surface glycoprotein GP46/M-2 of Leishmania amazonensis" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 87, November 1990 (1990-11), pages 8393-8397, XP002204079 ISSN: 0021-9258

D2: DUMONTEIL E ET AL: "DNA vaccines induce partial protection against Leishmania mexicana" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 21, no. 17-18, 16 May 2003 (2003-05-16), pages 2170-2177, XP004421134 ISSN: 0264-410X

D3: LEBOWITZ J H ET AL: "DEVELOPMENT OF A STABLE LEISHMANIA EXPRESSION VECTOR AND APPLICATION TO THE STUDY OF PARASITE SURFACE ANTIGEN GENES" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 87, December 1990 (1990-12), pages 9736-9740, XP002052133 ISSN: 0027-8424

D4: JIMENEZ-RUIZ A ET AL: "CLONING SEQUENCING AND EXPRESSION OF THE PSA GENES FROM LEISHMANIA INFANTUM" EUROPEAN JOURNAL OF BIOCHEMISTRY, BERLIN, DE, vol. 251, no. 1/2, 15 January 1998 (1998-01-15), pages 389-397, XP001159173 ISSN: 0014-2956

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Document D1 describes the cloning and the sequence of a *Leishmania (L.) amazonensis* surface glycoprotein which is immunoprotective in nature, in particular GP46/M-2. In view of figure 3 of the present application, certain protein regions of D1 are highly identical to the sequence corresponding to SEQ ID NO 6 (present application), in particular:

- 96.4% for the secretion pathway signal peptide (1-55:1-55);
- 93.5% for the region comprising the signal peptide (1-107;1-107);
- 83.3% for the leucine-rich repeat domain 1 (108-131:108-131);
- 87.5% for the leucine-rich repeat domains 4-6 (181-252:132-203);
- 100% for the poly P/T/S region (277-305:228-256); and
- 100% for the cysteine-rich region (306-339:257-290).

The major difference between the sequence corresponding to SEQ ID NO 6 and said sequence of D1 is therefore that it possesses two additional leucine-rich repeat domains and that the hydrophobic signal peptide is not highly identical.

Moreover, the sequence corresponding to

- SEQ ID NO 6 has an overall identity of 80% (93% ungapped) (1-339:1-290);
- SEQ ID NO 7 has an overall identity of 49% (60.44% ungapped) (*L. major* LmgSP5: AAB71312, not cited) (7-286:243-570);
- SEQ ID NO 8 has an overall identity of 69% (94% ungapped) (1-278:86-290);
- SEQ ID NO 9 has an overall identity of 92% (92% ungapped) (67-275:82-290);
- SEQ ID NO 10 has an overall identity of 68% (92% ungapped) (1-278:86-290); and
- SEQ ID NO 12 has an overall identity of 40% (60% ungapped) (1-445:1-306).

In view of the remarks made above, the proteins corresponding to SEQ ID Nos 6-10 and 12 can be considered to be variants of the protein disclosed in D1.

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

D2 describes the use of a cDNA encoding the *L. amazonensis* GP46/M6 protein for a vaccine. This DNA vaccine results in a partial protection against *Leishmania*.

D3 describes a genetically modified *Leishmania* strain transfected with an expression vector comprising a nucleic acid construct encoding the *L. amazonensis* GP46/M6 protein.
Furthermore, D3 alludes to variants of GP46 which can also vary by one or more modifications (D3, page 9740).

In addition, D4 reveals that the number of leucine-rich repeat domains of the GP46/M2 protein can vary from one *Leishmania* species to another and can also vary in the same species. Furthermore, the GP46/M2 protein can exist in soluble form and in membrane-anchored form.

1). The present application complies with the requirements of PCT Article 33(2) since the subject matter of claims 1-9 meets the requirement of novelty.

2). The present application fails to comply with the requirements of PCT Article 33(1) since the subject matter of claims 1-9 does not involve an inventive step as defined in PCT Article 33(3).

Document D3 is considered to be the prior art closest to the subject matter of claim 1.

Therefore, the subject matter of claim 1 differs from this known nucleic acid construct in that, in view of document D1, variants of nucleic acids encoding the known *L. amazonensis* GP46/M2 protein were used.

The problem that the present invention is intended to solve can thus be considered to be that of adding to the prior art other sequences encoding the known *L. amazonensis* GP46/M2 protein.

The solution, as proposed in claim 1 of the present application, is

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

not considered to be inventive (PCT Article 33(3)) for the following reasons:

Documents D3 and D4 disclose that variants of the known *L. amazonensis* GP46/M2 protein exists.

D1 describes that the known *L. amazonensis* GP46/M2 protein can be used to immunize mice, and document D2 describes the use of cDNA encoding the known *L. amazonensis* GP46/M2 protein as a DNA vaccine.

Because no surprising or new effect was described for the proteins which are encoded by a sequence chosen from SEQ ID Nos 1-5 and 11 in the present application, claim 1 does not involve an inventive step.

The same argument applies *mutatis mutandis* to the subject matter of the corresponding independent claims 6, 7 and 9, which are thus not inventive either.

Dependent claims 2-5 and 8 do not contain any feature which, in combination with the features of any one of the claims to which they refer, meets the requirements of the PCT in respect of inventive step; see documents D1-D4 and the corresponding passages cited in the search report.